THE FIREFLY PSEUDOFLASH IN RELATION TO PHOTOGENIC CONTROL [1]

J. WOODLAND HASTINGS AND JOHN BUCK

Department of Biological Sciences, Northwestern University, Evanston, Ill., and Laboratory of Physical Biology, National Institutes of Health, Bethesda 14, Md.

Introduction

The normal flashes of many fireflies are short sharp bursts of light lasting about a tenth of a second, with essentially total darkness between. The problem of how this light emission is so precisely controlled has long appealed to investigators interested in biological trigger mechanisms. Two basic facts have become firmly established: that nervous activity can initiate luminescence and that oxygen is essential for light production both in the intact firefly and in cell-free extracts (for review see Buck, 1948; Harvey, 1952; McElroy and Hastings, 1955; Buck, 1955).

There are two principal theories concerning the control of normal flashing. One postulates direct nervous stimulation of the photogenic cell, and the other proposes nervous control of the oxygen supply to the cell. There has been little empirical evidence bearing on the idea that the nerve impulse acts on the photogenic cell directly. The theory of oxygen limitation, on the other hand, has been widely supported on both physiological and anatomical grounds. Actually, as pointed out previously (Buck, 1948), all the experimental findings that have been ascribed to direct oxygen control can be equally well interpreted as effects on nervous control. The anatomical evidence is more persuasive, though circumstantial. It consists of the facts (a) that tracheal end cells under the light microscope appear to have a structure which can be interpreted as valvular (Dahlgren, 1917), (b) that end cells are present in the photogenic organs of flashing types of fireflies and absent in types that produce only sustained glows, and (c) that the end cells are strategically situated on the tracheae at the points where the tracheoles enter the photogenic tissue.

The principal experimental support for the idea that the end cell functions as a valve comes from the work of Snell (1932) and Alexander (1943). They found that fireflies exposed to low oxygen concentrations developed a dull "anoxic glow" (= our "hypoxic glow"), and when suddenly re-exposed to air produced a brilliant "pseudoflash" lasting a second or more. They interpreted these events as follows: The low oxygen narcotizes the normally closed end cell valves and causes them to open, allowing the ambient gas to enter the previously anaerobic photogenic cytoplasm. Since the oxygen concentration is low, only a dull glow develops. When air is subsequently admitted the higher oxygen concentration permits a more brilliant luminescence, which, however, then quickly dies out as the valves recover and close in the higher oxygen concentration. From these hypoxic glow-pseudoflash responses it was argued that normal flashing could also be controlled by end cell limitation of oxygen. It should be noted, however, that all the experiments in-

[1] This work was supported in part by a grant from the National Science Foundation.

volved markedly unnatural conditions and hence are not necessarily relevant to normal flash control.

Recent experiments of McElroy and associates on cell-free extracts of firefly photogenic tissue appear to bear directly on the question of oxygen limitation versus nerve stimulation. When all the components required for light emission (luciferin, luciferase, Mg^{++}, adenosine triphosphate and oxygen) are mixed together a flash of light occurs, its intensity reaching a peak in less than 0.1 second and then declining to a low sustained level within the next 10–20 seconds. Evidence too detailed to present here indicates that this *in vitro* flash involves several reactions (McElroy and Hastings, 1955; McElroy, personal communication). First, it is believed, luciferin, luciferase and ATP react to form a luciferin-adenylic acid-enzyme "active intermediate." The intermediate is then oxidized rapidly and irreversibly, with emission of light. This oxidation corresponds to the initial rise of luminescence in the flash. However, the oxyluciferin formed during luminescence undergoes a slower, reversible phosphorylation by ATP to form oxyluciferin-adenylic acid, which strongly inhibits the enzyme in the active intermediate. This inhibition accounts for the decline in light intensity after the initial peak—a decline which takes place in the presence of excess oxygen. The eventual low level plateau of luminescence thus reflects the low concentration of uninhibited enzyme available once a steady state among the various reactions is established.

The concentration of the active intermediate—which may be considered to be the substrate of the light-producing reaction—can be changed in two significantly different ways: (1) If oxygen concentration is greatly decreased the rate of the oxidative reaction is decreased, resulting in decreased luminescence and accumulation of active intermediate. When air is readmitted the accumulated intermediate is rapidly oxidized, resulting in a flash of light. This shows that it is possible, by changing oxygen concentration, both to limit luminescence and to cause a flash. The "oxygen flash" of the extract has a remarkable quantitative resemblance to the pseudoflash of the intact firefly, which, it will be remembered, is induced by a similar sequence of changes in oxygen concentration. This suggests that both types of flash are due to oxidation of accumulated active intermediate, and that neither of them needs be oxygen-limited during its decay phase. (2) The concentration of the active intermediate may also be increased by addition of pyrophosphate, which, by opposing the formation of the oxyluciferin-adenylic acid inhibitor, frees active enzyme. Since this reversal of enzyme inhibition is rapid, lasts only until the added pyrophosphate is used up, and does not involve any change in oxygen concentration, it provides a possible model for the mechanism which induces the normal flash.

The *in vitro* system therefore suggests that the flashing of the firefly need not normally be controlled by oxygen concentration even though light production may, under some artificial conditions, become oxygen-limited. In view of this possibility, and of the ambiguity of previous oxygen-limitation experiments on intact fireflies, it is important to re-examine the evidence purporting to demonstrate end-cell control of luminescence.

Materials and Methods

The forms investigated were adults of the lampyrid fireflies *Photuris* sp. and *Photinus pyralis* from the Baltimore-Washington area, adults of the elaterid firefly

Pyrophorus atlanticus from Florida, and larvae of *Photuris*. In the males of the first two species, as in many lampyrid fireflies, the photogenic tissue occupies the ventral surfaces of abdominal segments 6 and 7. In *Pyrophorus* we investigated the small circular organs at the posterior dorsal corners of the prothorax. In the *Photuris* larva the photogenic organs are a pair of small lateral plaques on the ventral side of abdominal segment 8. Similar organs exist in the larva and pupa of *Photinus pyralis*, and sometimes persist into the adult where they function independently of the main organs.

Different gas mixtures were prepared by passing various gases through calibrated flow meters into a mixing chamber and thence to the exposure chamber, which was a 5 cm. length of glass tubing of 6 mm. bore. For visual observation up to three specimens were accommodated in the chamber, separated by wire screen partitions. A flow rate of 300–400 mL per minute was used and a reversing stopcock between mixing and exposure chambers permitted quick shifting from one gas to another, the unused gas being vented. Light intensity was measured with a photomultiplier tube apparatus (Hastings, McElroy and Coulombre, 1953) and recorded with an oscilloscope camera or a graphic meter, the firefly being held in position against the wall of the exposure chamber with a loose cotton plug.

Results

1. Responses of intact lampyrid fireflies to varied oxygen concentration

When adults of *Photinus* and *Photuris* were exposed to various low oxygen concentrations a dim hypoxic glow usually developed, and when such glowing fireflies were suddenly re-exposed to air a pseudoflash occurred. Figure 1 illustrates a response of this sort, the hypoxic glow being represented by BC and the pseudoflash by CDE. Figure 2 shows the pseudoflash portion in more detail. An "oxygen flash," which occurs when air is readmitted to an anaerobic cell-free extract of photogenic tissue, is reproduced in Figure 3 for comparison. About 50 records of the hypoxic glow and pseudoflash have been made and analyzed, supplemented by many hundred visual observations. These in general confirm the findings of Snell and of Alexander, but certain differences were noted. For example, if a firefly was left in the low oxygen mixture after the hypoxic glow had reached its plateau level, instead of then exposing it to air, the intensity of the glow usually decreased over the course of several minutes (Fig. 1, dotted line, FG). It was also observed that some individuals failed to give a pseudoflash, or both hypoxic glow and pseudoflash, and that there was considerable variation in the length of the period between hypoxic exposure and beginning of the hypoxic glow (Fig. 1, AB), and in the intensities of both glow and pseudoflash. Also, as in nature, normal individuals sometimes showed an initial constant dim glow in air which made it difficult to recognize the start of the hypoxic glow.

In spite of these variations in response, a number of quantitative relations were apparent. First, in fireflies which had been exposed to a particular low oxygen gas mixture, the peak intensity of the pseudoflash in air was approximately proportional to the intensity of the hypoxic glow in the low oxygen mixture just prior to admission of air. Before the beginning of the hypoxic glow no pseudoflash could be elicited; during the dimmer periods of the hypoxic glow, either before or after the maximum, pseudoflashes of low intensity occurred; and during the brighter pe-

riods of the hypoxic glow more brilliant pseudoflashes occurred. In 35 experiments in which the hypoxic glow was induced with 0.25% oxygen, the ratio of pseudoflash intensity to hypoxic glow intensity varied only from 30 to 120, and the range for repeated measurements on single individuals was even smaller. Thus both pseudoflash and hypoxic glow intensities pass through a maximum with time.

A second finding was that the ratio of pseudoflash intensity to hypoxic glow intensity varied with the oxygen concentration used to induce the hypoxic glow. For example, in one typical individual the pseudoflash intensity in air was 1.5 times

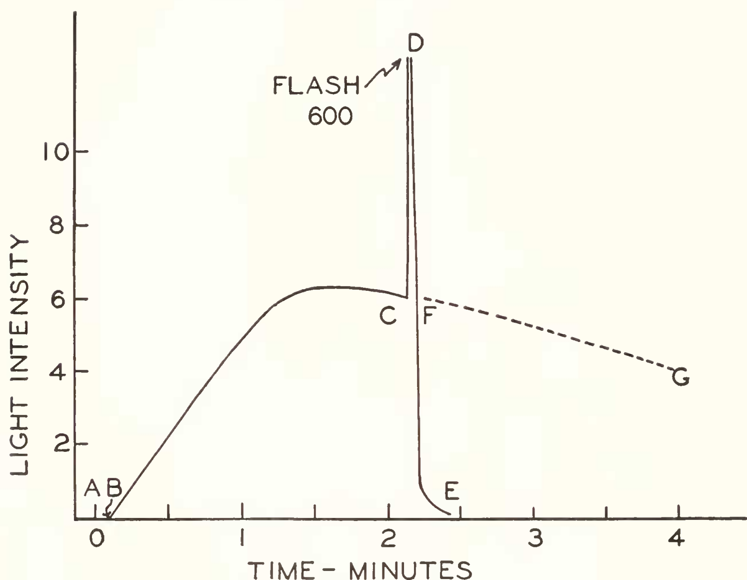
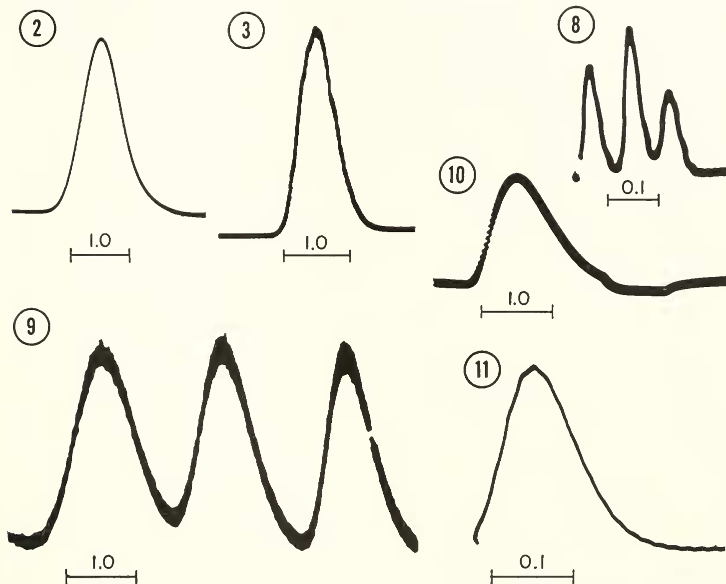


FIGURE 1. Hypoxic glow-pseudoflash response of intact male of *Photinus pyralis*, diagrammed from graphic meter record. Ordinate, light intensity in arbitrary units. At A (zero time) ¼% oxygen was introduced. At B the hypoxic glow began, AB representing the latent period. At C air was flushed through the chamber and a pseudoflash of 600 units relative intensity occurred. Had the firefly been left in ¼% oxygen at C, the hypoxic glow would have continued (FG), slowly diminishing in intensity.

as great as the hypoxic glow elicited with 2.4% oxygen;[2] when 1% oxygen was used the pseudoflash was 10 times as bright as the glow; when 0.25% oxygen was used the pseudoflash was 60 times as bright; and when 0.05% oxygen was used the pseudoflash was 5000 times as bright. The increase in this ratio with progressively lower oxygen concentrations was evidently due both to diminution in hypoxic glow intensity and to an increase in the absolute pseudoflash intensity.

[2] No systematic attempt was made to find the upper oxygen concentration limit for pseudoflash occurrence, but it is certainly higher than the limit set by Snell (about ½%).

A third characteristic of the hypoxic glow-pseudoflash response is that the pseudoflash was remarkably constant in duration and in form (rates of accretion and decay of intensity), regardless of variations in both degree and duration of hypoxia prior to readmission of air. This was true both in repeated measurements with one individual and in records from different individuals. Intensity variations of well over a thousand-fold occurred without difference in duration. If the pseudoflash were being controlled by some sort of oxygen-sensitive effector, such as Snell



FIGURES 2, 3, 8, 9, 10, 11. Various luminous responses photographed from the oscilloscope screen. Ordinate, light intensity; abscissa, time, with sweep going from left to right. Further descriptions in text. Time scale in seconds. FIG. 2. Pseudoflash of intact adult male of *Photinus pyralis*. FIG. 3. Oxygen flash of cell-free extract of *P. pyralis*. FIG. 8. Spontaneous flashing of intact female of *Photuris*. FIG. 9. Spontaneous glow of intact *Photuris* larva. FIG. 10. Pseudoflash of *Photuris* larva. Temporary depression in trace following flash represents period during which photocell power supply was switched off. FIG. 11. Spontaneous flash of intact male of *P. pyralis*.

supposed the end cell to be, it would be remarkable that this degree of constancy of response could be achieved, particularly in view of the individual variability in intensity and latency of hypoxic glow, and in intensity of pseudoflash.

2. Oxygen responses in relation to structure

It was shown previously (Buck, 1948) that there is no correlation between the state of spiracular valves and the times of occurrence or characteristics of the normal

flash of *Photinus pyralis,* and that when intact fireflies are tested with progressively falling oxygen concentration the spiracles open well before the hypoxic glow begins and close after it ceases. Evidence that the spiracles have no immediate influence on the hypoxic glow-pseudoflash response was obtained in the present study by testing adults of *Photuris* and *Photinus* in which the spiracles of the luminous segments had been made inoperative by cautery with an electrically heated needle or by insertion of a short length of human baby hair. Although these specimens often showed a continuous glow in air, presumably caused by the mechanical dis-

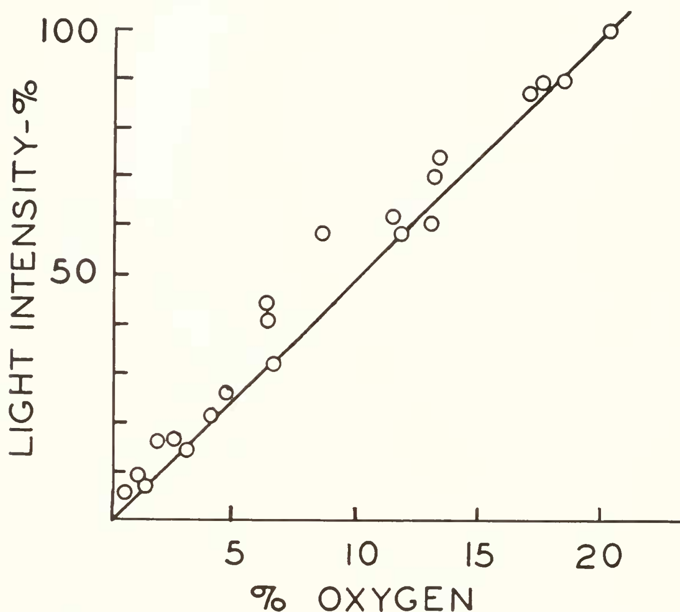


FIGURE 4. Relation between oxygen concentration and glow intensity (in per cent of intensity in air) of smeared photogenic tissue of males of *P. pyralis.* Data from several experiments.

turbance, they gave pseudoflashes similar to those in individuals with normal spiracles. Likewise, it was observed that spiracular opening is regularly induced by exposure to 5% oxygen, whereas the hypoxic glow usually requires that the ambient oxygen concentration be reduced to the order of 1% to 2%. Absence of spiracular or indeed any sort of valvular influence is also seen in dead fireflies which, if prevented from drying out, may exhibit a constant dim air glow for a day or more after all visible signs of life have disappeared. Such dead specimens have permanently open spiracles yet give a pseudoflash response.

A series of experiments was performed in which increasing degrees of interfer-

ence with possible central nervous or tracheal control of luminescence were achieved by (a) decapitation, (b) cutting off the abdomen at the junction of the fifth and sixth segments, (c) excising the photogenic organ alone, and (d) smearing the photogenic tissue on glass. None of these preparations produced normal flashes, or indeed any continued spontaneous luminescence, except for the smeared organs, which exhibited a continuous dim glow in air, decreasing in intensity very gradually

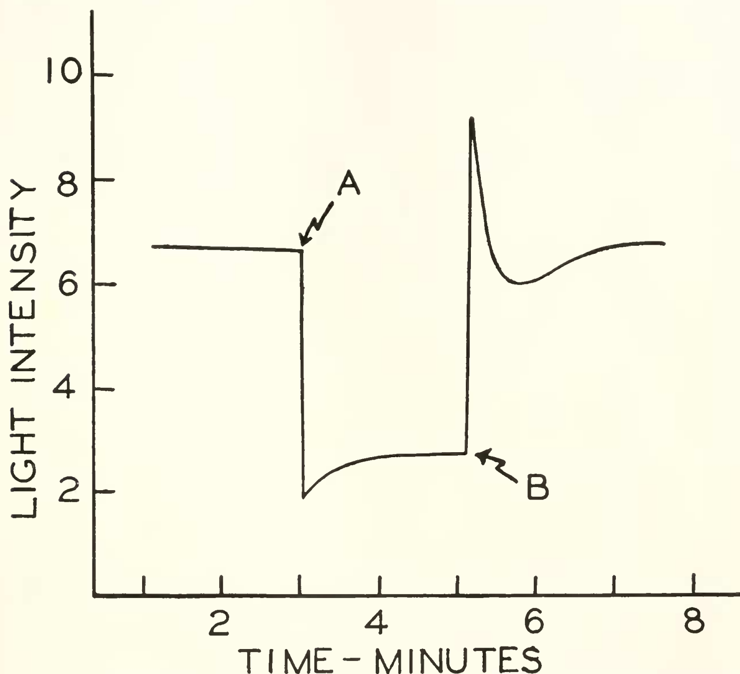


FIGURE 5. Changes in light intensity of smeared photogenic tissue of male of *P. pyralis* in varied oxygen concentrations. Tissue in air for first three minutes. At A, 8.3% oxygen was introduced. At B, air was readmitted, inducing a pseudoflash-like excess luminescence.

(30 minutes or more to extinction). The first three types of preparations responded to changes in ambient oxygen concentration just like intact fireflies, *i.e.*, in low oxygen concentrations they developed hypoxic glows and when air was readmitted they produced typical pseudoflashes. It is thus clear that nerve impulses originating in the central nervous system play no role in the photogenic response to hypoxia. It is also apparent that none of the tracheae external to the light organ is involved.

The intensity of the glow of the smeared organ is proportional to ambient oxy-

gen concentration below 21% (Fig. 4) and also increases greatly in pure oxygen. Figure 5 shows the time course of the luminescence when a smeared organ was exposed (at A) to 8.3% oxygen and then re-exposed (at B) to air. The changes in luminescence are qualitatively very similar to those which occur when the same procedure is carried out with cell-free extracts (Hastings, McElroy and Coulombre, 1953). The smeared preparation differs from both the extracts and the intact organ in that its pseudoflash has a longer duration and is not so bright relative to the

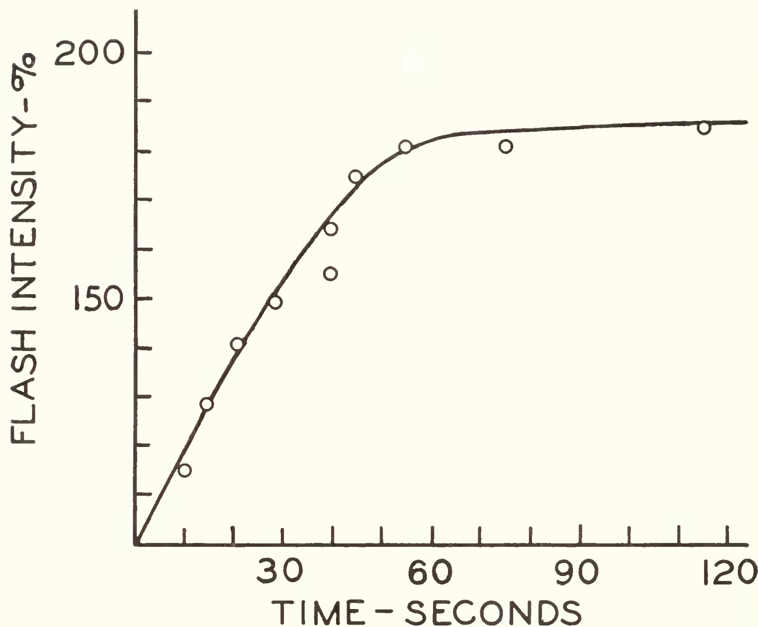


FIGURE 6. Relation between duration of hypoxia and intensity of pseudoflash in air of smeared photogenic tissue of *P. pyralis*. Each point represents pseudoflash intensity, in per cent of glow intensity in air, in an experiment similar to that diagrammed in Figure 5. The hypoxic mixture used contained 1% oxygen.

intensity of the hypoxic glow. These differences in glow and flash are to be expected if oxygen has become limiting in the luminescent reaction, and presumably it is the disruption of the tracheal supply within the photogenic tissue itself which is responsible for this oxygen-limitation. Similar "slow" flash responses have been demonstrated in extracts under conditions of oxygen limitation (McElroy and Hastings, unpublished). On the basis of the biochemical reactions already discussed, the intensity of the pseudoflash of the smeared organ is presumably a measure of the amount of active intermediate which has accumulated. The de-

pendence of this accumulation upon both time of hypoxia and oxygen concentration in the hypoxic gas mixture is illustrated in Figures 6 and 7.

In normal males of *Photinus* and *Photuris* all the photogenic tissue in both luminous segments ordinarily participates in each flash, and apparently simultaneously.

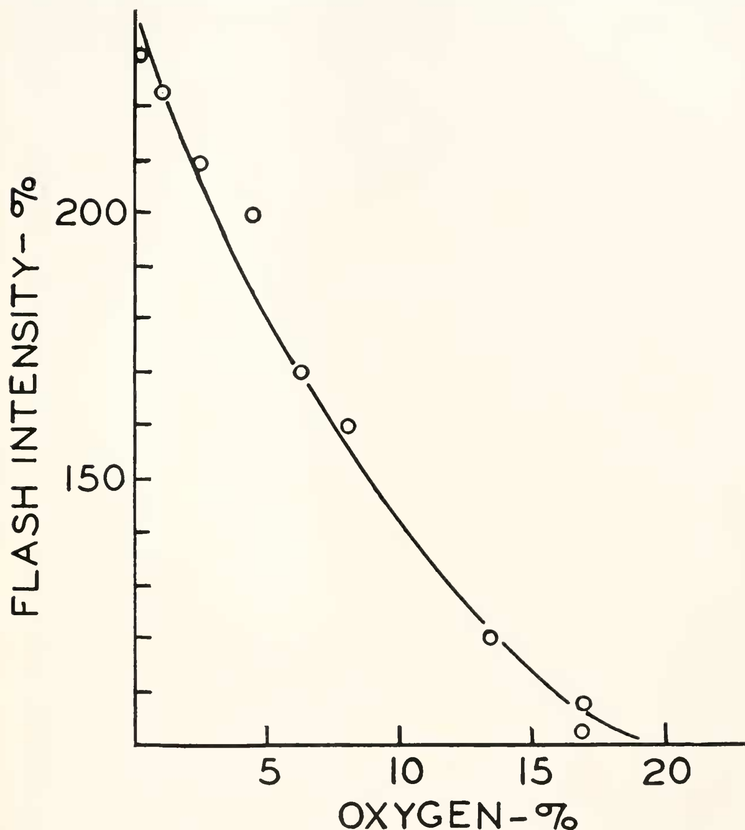


FIGURE 7. Relation between intensity of pseudoflash in air of smeared photogenic tissue of *P. pyralis,* and degree of hypoxia. Tissue exposed to each oxygen concentration for two minutes, then flushed with air. Intensity plotted on ordinate in per cent of glow intensity in air.

In some instances, however, it was observed that both spontaneous flashes and various types of induced luminescence involved only one of the segments, or only parts of one or both. Furthermore, the type of luminescence displayed sometimes differed in different regions of a single organ or even changed in the course of an experiment.

The flash of the female of *Photuris* is usually too sharp and brilliant for reliable visual observation of heterogeneity, but may possibly also involve asynchronous luminescence (Fig. 8).

In instances in which only a portion of a photogenic organ gave the hypoxic glow-pseudoflash response, the portion which failed to respond often developed a dull glow in air after the pseudoflash in the other portion had ceased. Since the photocell integrates all the light emitted, one needs to be aware of the possibility that intensity × time recordings of luminescence (*e.g.,* Figs. 1, 2) may be the resultant of two quite different sorts of things, namely, change in light intensity per unit organ area, and change in area active. We cannot exclude the possibility that an occasional heterogeneous response of this type was recorded in our work, but do not believe that any of our present interpretations is in error because of such an accident.

3. *Oxygen effects on induced glows*

Bright steady glows can be induced in both intact fireflies and isolated abdomens by air passed through cotton soaked in ethyl ether or over potassium cyanide crystals (*i.e.,* without change in ambient oxygen concentration). The dosage must be chosen to avoid either premature recovery of the animal or rapid destruction of the photochemical system (Buck, 1948). When fireflies which were glowing from exposure to ether or cyanide vapor were exposed to low oxygen concentrations, luminescence abruptly declined to a low level, then rose somewhat as an hypoxic glow. When air was readmitted a typical pseudoflash occurred. This illustrates the occurrence of a flash under conditions in which tracheal end cells would be expected to be inactivated.

4. *Oxygen responses of Pyrophorus, Photuris larva and persistent pupal organ of Photinus pyralis*

The photogenic organs of the large elaterid firefly *Pyrophorus* and of the larvae and pupae of lampyrid fireflies offer an interesting contrast to the organs of adult lampyrid fireflies in two respects. First, they never normally flash, but emit light in long-sustained glows at irregular intervals. Second, they lack the tracheal end cells which are characteristic of the flashing-type adult lampyrid organ. The pupal organs frequently persist into the adult, thus combining both organ types in the same individual.

Observation of oxygen effects in *Pyrophorus* is complicated by the fact that the glow normally fluctuates cyclically in intensity, at frequencies varying from about one peak per second to one per five seconds or slower, as observed also in a Cuban species by Harvey (1931). In addition, the intensity of the glow increases markedly when the creature is disturbed. Thus hypoxia sometimes proves sufficiently irritating that the light emitted is at first actually brighter than in air, and there is no initial decline due to oxygen limitation as in adult lampyrids glowing in air. The luminescence is only oxygen-limited at ambient concentrations of 1% or lower. *Pyrophorus* in low oxygen concentrations responds to a sudden increase in oxygen by emitting a pseudoflash which is qualitatively similar to the typical adult lampyrid pseudoflash, but often appears after a quite long latent period (up to 20 seconds) and lasts much longer.

The minute photogenic organs of the larva of *Photuris* and the persistent pupal organs of *Photinus pyralis* emit a fluctuating luminescence strikingly similar to that of *Pyrophorus* (Fig. 9). Their responses to oxygen have not been followed in detail except to confirm Buck's observation (1946, 1948) that low ambient oxygen induces an hypoxic glow and subsequently raised oxygen elicits a pseudoflash. A record reproduced in Figure 10 shows that the larval pseudoflash closely resembles that of the adult. The persistent pupal organ in the adult usually (but not always) gives an hypoxic glow-pseudoflash response in parallel with that of the adult organ.

Discussion

Evidence presented above has shown that neither spiracle, main trachea nor central nervous system is necessary for either the appearance or disappearance of luminescence in the usual type of hypoxic glow-pseudoflash response. The possibility that tracheal end cell valves might be involved in the response is likewise all but eliminated by the following considerations: (a) Pseudoflashes occur in *Pyrophorus*, the *Photuris* larva and the persistent pupal organ of *Photinus pyralis* (all of which lack end cells), and in lampyrid fireflies treated with cyanide and ether (where end cell valves should be inactivated); (b) as already pointed out, the constancy of pseudoflash duration makes it difficult to believe that an end cell mechanism is functioning in the control; (c) recent electron microscopy by Beams and Anderson (1955) casts grave doubt on there being any valvular structure in the end cell. In fact the induction of pseudoflashes in dead fireflies makes it unlikely that this response depends upon active participation of any part of either tracheal or nervous systems.

Even with end cell control excluded there remains the question of whether the hypoxic glow-pseudoflash response might nevertheless be controlled by oxygen limitation. We have seen that in the glow of smeared tissue and, within a narrow concentration range, in the hypoxic glow itself, oxygen does appear to be a limiting reactant. However, the hypoxic glow is actually induced not by increase in oxygen concentration but by decrease, and the pseudoflash dies away (*i.e.*, is controlled) under conditions in which ambient oxygen concentration, if changing at all, must be rising. When we add to these paradoxes the fact that glowing can be induced by pure oxygen (Alexander) and by a wide variety of physical and chemical agencies, and that even the hypoxic glow can change spontaneously in intensity without any change in ambient gas concentration, it becomes very difficult to visualize oxygen as playing any consistent role in either initiating or stopping these induced types of luminescence.

For reasons discussed by Buck (1955) the striking kinetic similarity between pseudoflash and oxygen flash (Figs. 2, 3) does not necessarily indicate the same causation. However, the detailed parallels between the two responses leave little doubt that the pseudoflash of the intact organ involves the photochemical system identified in the cell-free extract. Thus active intermediate can be presumed to accumulate in the photogenic tissue during hypoxia, and, upon readmission of air, to be concurrently oxidized and inhibited with production of a pseudoflash (see Introduction). Similarly, assuming that liberation of active intermediate would continue in the organ of a dead firefly until autolysis supervened, the ability of some dead individuals to glow and to give pseudoflashes could be explained. Furthermore, the *in vitro* system is free from all the morphological objections to end cell

involvement discussed above and it is consistent with the constant duration of the pseudoflash, which is particularly difficult to account for on the basis of valvular control. Such constancy, in other words, is precisely what would be expected if the luminescence decays primarily because of an enzyme-inhibiting reaction rather than because of oxygen limitation.

The conclusion that no end cell valve functions in the hypoxic glow-pseudoflash response does not, of course, exclude the possibility that normal flashing is controlled by such a mechanism. Since, however, the induction of the pseudoflash has been the principal experimental support for belief in end cell control, the existence of a more reasonable alternate explanation of the pseudoflash leaves little ground for favoring end cell involvement in the normal flash. Furthermore, oxygen control by whatever method appears intrinsically less tenable than enzymatic control on at least two counts: First, if the flash were oxygen-limited the photogenic tissue would have to be hypoxic throughout the long interflash periods. This would be unlikely on physiological grounds, even if respiration had a lower oxygen requirement than luminescence (actually, in all forms thus far studied luminescence persists at oxygen concentrations far lower than will support any significant respiration). If, however, the normal flash were controlled by temporary reversal of enzyme inhibition, rather than by oxygen limitation, the tissues could remain fully aerated at all times. Second, the normal flash of many lampyrid fireflies is so short as to cast doubt on the possibility of control by diffusive gas transfer. Even in the relatively slow flash of *Photinus pyralis* (Fig. 11) the average rise time is 0.075 second, and the response of individual photogenic units is almost certainly much faster (Buck, 1948, p. 446). In the female of *Photuris* (Fig. 8) the rise time is not more than 0.03 second and the decay of luminescence is almost equally rapid. This shows that an efficient mechanism for oxygen removal would need to be present as well as one for suddenly supplying oxygen. *A priori*, therefore, it would be expected that an intracellular mechanism involving enzyme inhibition and activation would be better suited to the required response velocities than one involving passage of oxygen between tracheae and cell.

The fact that intact fireflies are able to extinguish their light completely between flashes, whereas low-level luminescence continues in the *in vitro* system, need not be unduly disturbing since the intact cell presumably has more efficient methods of shifting the chemical equilibria concerned and of sequestering reactants. It has been suggested (Buck, 1948, 1955) that the photogenic tissue is ordinarily kept dark by some sort of aerobic metabolic process and that light is produced only when this process is interfered with. Such a mechanism would account for both "abnormal" and normal luminescence, since it should be inhibited by very diverse agencies such as hypoxia, various poisons (*e.g.*, pure oxygen, cyanide) and anesthetics (ether)—allowing light to be produced—while at the same time forming a likely type of system to be integrated with the normal biological trigger, the nerve impulse. A tentative biochemical pathway has recently been suggested (McElroy and Hastings, 1955) by which a nerve impulse might lead to a rapid temporary increase in active intermediate concentration—*i.e.*, to a flash. Whether or not the precise mechanism suggested proves to be correct, this general type of linkage of stimulatory and response systems deserves special attention because it provides an endogenous mechanism capable of the observed rapidity and precision of photogenic control.

Summary

As reported by Snell and Alexander, lampyrid fireflies exposed to oxygen concentrations of the order of 2% or lower develop a sustained "hypoxic glow," and when subsequently re-exposed to air emit a much brighter and shorter "pseudoflash." We find that these responses can be independent of the spiracles, and are given by decapitated fireflies, isolated abdomens and excised photogenic organs, showing their independence of central nervous system and tracheae. The hypoxic glow-pseudoflash response is also given by the elaterid firefly *Pyrophorus* and by the larval and pupal photogenic organs of lampyrid fireflies. Since all these organs lack tracheal end cells, these cells cannot, as Snell and Alexander believed, control this type of light production. This, together with other evidence, makes it clear that luminescence is rarely oxygen-limited. Rather, all our observations are consistent with enzyme activation and inhibition in a system of photochemical reactions of the sort proposed by McElroy and Hastings (1955).

LITERATURE CITED

Alexander, Robert S., 1943. Factors controlling firefly luminescence. *J. Cell. Comp. Physiol.*, **22**: 51–71.

Beams, H. W., and Everett Anderson, 1955. Light and electron microscope studies on the light organ of the firefly (*Photinus pyralis*). *Biol. Bull.*, **109**: 375–393.

Buck, John B., 1946. The spiracular factor in the control of luminescence in the firefly. *Anat. Rec.*, **96**: 51.

Buck, John B., 1948. The anatomy and physiology of the light organ in fireflies. *Ann. N. Y. Acad. Sci.*, **49**: 397–482.

Buck, John, 1955. Some reflections on the control of bioluminescence. Pp. 323–332 in The Luminescence of Biological Systems (Ed. F. H. Johnson), Amer. Assoc. Adv. Sci., Washington.

Dahlgren, Ulric, 1917. The production of light by animals. *J. Franklin Inst.*, **183**: 323–348.

Harvey, E. N., 1931. Photocell analysis of the light of the Cuban elaterid beetle, *Pyrophorus*. *J. Gen. Physiol.*, **15**: 139–145.

Harvey, E. N., 1952. Bioluminescence. Academic Press, New York.

Hastings, J. Woodland, William D. McElroy and Jane Coulombre, 1953. The effect of oxygen upon the immobilization reaction in firefly luminescence. *J. Cell. Comp. Physiol.*, **42**: 137–150.

McElroy, W. D., and J. W. Hastings, 1955. Biochemistry of firefly luminescence. Pp. 161–198 in The Luminescence of Biological Systems (Ed. F. H. Johnson), Amer. Assoc. Adv. Sci., Washington.

McElroy, W. D., J. W. Hastings, Jane Coulombre and Valerie Sonnenfeld, 1953. The mechanism of action of pyrophosphate in firefly luminescence. *Arch. Biochem. Biophys.*, **46**: 399–416.

Snell, Peter A., 1932. The control of luminescence in the male lampyrid firefly, *Photuris pennsylvanica*, with special reference to the effect of oxygen tension on flashing. *J. Cell. Comp. Physiol.*, **1**: 37–51.